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Textural Properties of Casein(ate) Based Mixed Gels for Use as Surimi-like Seafood Analogues

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ABSTRACT

The wide range of physicochemical and functional properties of caseins and caseinates, as well as their bland flavor, make them an excellent protein source for the creation of formulated foods. Their ability to gel suggests potential use in highly valued imitative products, such as Kamaboko, a surimi product. Gelation, and the resultant textural properties, can be enhanced by various factors such as ionic strength, addition of non-casein proteins or the use of additives such as polysaccharides. The addition of κ -carrageenan (1 to 2% wt/wt) and sodium hexametaphosphate to calcium caseinate (25 to 30 % wt/wt), followed by a high shear mixing and heat treatment at 80 °C, was effective in producing a gel with markedly improved elasticity and water holding capacity. The creation of mixed protein gels, using caseinate and egg albumin, was also effective in producing a Kamaboko-like product at significantly lower protein concentrations.

INTRODUCTION

Caseins, a family of phosphoproteins present in milk, are desirable functional ingredients in a wide variety of foods including coffee creamers, confections, extruded snacks, cheese analogues, and meat products (Southward, 1989). The functional attributes that make them valuable as food additives include the enhancement of texture and body, good foaming and fat emulsification and high water holding capacity. The bland flavor of these proteins increases their utility even further. Although much is known about casein chemistry, fundamental data on the properties that govern functionality, particularly in the presence of other food components, are somewhat limited (Kinsella, 1984).

Caseins in milk form gels when subjected to specific treatment such as acid (Parnell-Clunies et al., 1988a), heat (Parnell-Clunies et al., 1988b), aging (Manji and Kakuda, 1988), and proteolysis (rennet) (Dalglish, 1982). These gels form when the native quaternary structure of casein (casein micelle) is partially disrupted and the micelles join together forming the gel structure. Caseins and caseinates, in the dried powder form used as food additives or ingredients, do not readily form gels. However, when combined with low concentrations of polysaccharides and other proteins, the resultant gels can have a wide variety of rheological and textural properties that can be utilized for the creation of novel or imitative foods. The rational, methodology, development, and textural evaluation of a casein-based surimi type analogue (Konstance, 1994) will be described and discussed.

A seafood analogue made from casein or caseinate has several advantages. Since casein is a milk protein, it is a relatively easily replenished source; whereas fish protein is dependent on what is becoming a depleted resource (Holmes et al., 1992). Unlike the surimi-based seafood analogue, where large quantities of sodium chloride are required in the mincing/washing process (Konstance, 1994), the casein(ate) based analogue contains little sodium.

Kamaboko, frequently referred to as steamed fish cake, is a typical Japanese seafood whose

origin dates to the twelfth century. It was originally developed as a method of preserving the gelling properties of washed, minced fish (Suzuki, 1981) or what we know today as surimi. Kamaboko is a homogeneous protein gel made from fish myofibrillar protein, primarily actomyosin, that has been washed to remove the water soluble protein, ground with sodium chloride to solubilize the actomyosin and heated to form a gel. The frozen surimi of Alaska Pollock is the major source in the creation of kamaboko because of its relative availability and acceptable gelling properties (Mitchell, C.K., 1984). The unique characteristics of kamaboko are its cohesiveness and highly elastic texture as well as the strength of its gel.

Polysaccharide additives are often used to enhance structure by thickening and stabilization and gelation (Stanley, 1990). Potato starch is used to increase the rigidity of surimi gels (Lanier, 1986) and carrageenans are frequently added to dairy products such as ice cream, custards and chocolate milk to increase viscosity. Moderately high concentrations of κ -carrageenan cause casein to gel (Fox and Mulvihill, 1990). The effects of additions of potato and wheat starches and κ -carrageenan on the texture of casein-surimi analogue were examined.

Interaction of two or more proteins can occur in a complex food system with synergistic enhancement of textural properties of resultant gels. Egg albumin (EA) and whey protein concentrate (WPC) increase the hardness and elasticity of surimi gels made from Pacific Whiting (Chang-Lee et al., 1990). In the cheese-making process, the interaction between β -lactoglobulin (a whey protein) and κ -casein during heating profoundly affects the characteristics of the casein gel (Schmidt, 1979). The textural properties of gels made with combinations of EA and WPC with casein were examined.

Ionic strength of the protein sol or solution is also known to have profound effects on the textural properties of the resultant gels. Accordingly, the effects of polyphosphates on the texture of the casein-surimi analogue and the effects of calcium ion on texture of casein gels were examined.

METHODOLOGY

Sample Preparation

Kamaboko loaves used for this study were the "Itatsuki" variety made from Alaska Pollock and purchased locally. The loaves were sliced to the desired height (15 mm) using a parallel wire slicer. Cylindrical samples were extracted from the slices using a 15 mm diameter #11 cork borer. To minimize sample deformation during slicing and cylinder extraction, all sampling was accomplished at 4°C. Length to diameter ratios, (L/D) \geq 0.95, were used for all analyses. Samples were rejected when major flaws (air pockets on surface) were detected. Samples analyzed at temperatures other than 25 °C were maintained in sealed containers in a water bath.

Caseinate/Polysaccharide Gels

Caseinate with added polysaccharides (starches, carageenans) were prepared by mixing the protein powder and the various additives with crushed ice. The mixing method used (Strange and Konstance, 1991) provides for rapid and uniform dispersion utilizing a Cuisinart (Stamford, CT) food processor (750 mL bowl capacity) equipped with a stainless steel blade rotating at 1790 rpm. All samples were mixed for 10 min., removed from the food processor and spooned into twirl bags. The mixtures were compacted into the bags before sealing and placing in a 90 °C water bath. Samples were heated for 1 h, creating a uniformly viscous sol, removed from the water bath and allowed to gel under refrigerated conditions overnight. Samples for the Instron analyses were created as described above.

Mixed Protein Gels

Casein was prepared from fresh raw milk by acid precipitation with 1 N HCl, filtered and stored frozen. The mixed protein gels were prepared from stock solutions of the individual proteins. Thawed casein was resuspended in a minimum of water and adjusted to pH 7.0 with 50% NaOH. Aliquots were diluted to 7.5, 10 and 12.5 % for controls. Dilutions of 5% also were prepared to be mixed with solutions of egg albumin (EA) or whey protein concentrate (WPC). Final concentrations of mixtures were 2.5% casein with 5, 7.5 or 10 % EA or WPC. Each mixture was divided into three 80 ml flasks; sufficient 1 M CaCl_2 was slowly added during stirring for final concentrations of 10, 20 and 40 mM Ca^{2+} and stirred for about 5 minutes. 1.6 ml of 1:500 diluted commercial rennet was pipetted into each solution of a second experimental set, stirred at moderate speed for 1 min. at 25 °C and allowed to set for 30 min. Both sets were heated in a water bath at 80 °C for 30 min., briefly chilled, centrifuged at 37 °C for 30 min. at $83,500 \times g$, decanted, and drained inverted for 5 min. and the solids were refrigerated until preparation for textural analysis.

Cylindrical samples of the gels, 10 mm in diameter, were removed directly from the test tubes using a #7 cork borer. Direct removal of the samples from the tubes, because of the structural support and confinement of the gels, improved the cylinder geometry. Cylinders that were solid and did not slump were then cut to a 10 mm height. Kamaboko samples of similar dimensions were prepared for comparative purposes.

Rheological Analysis

Instrumental texture profile analyses (ITPA) double compression, were used to determine the rheological responses of the kamaboko and casein(ate) gels. Analyses were conducted using an Instron Universal Testing Machine (model 4201; Canton, MA) with 5.6 cm Lucite plates. Instron control was maintained using a series XII Cyclic Test application program. A 500 Newton load cell was used for all analyses. Samples were compressed to 50 % (or other selected values) at a constant rate of 50 mm/min. All samples were tested at 25 °C. Stress-strain data were derived from the force-deformation data (Instron output) using the following equations

$$\text{Hencky Strain} = \ln [h_0 / (h_0 - \Delta h)] \quad (1)$$

and

$$\text{True Stress (MPa)} = F_t / A_0 \times [(h_0 - \Delta h) / h_0] \quad (2)$$

where h_0 = original sample height in mm, Δh = change in height (mm) during testing, F_t = compressive force in Newtons at time t and A_0 = original cross-sectional area of the sample.

Instrumental Texture Profile Analysis Parameters

The Instrumental Texture Profile Analysis was selected for several reasons. ITPA is a widely accepted method for texture evaluation of a variety of foods and it is an imitative test that has been shown to correlate very well with the sensory texture profile data. Second, it provides one of the more precise definitions of overall response to the material. Third the ITPA is a good measure of binding or cohesive properties of gel materials. The ITPA parameters, determined from the force-deformation curve shown in Figure 1, were: *Hardness* (the force in Newtons necessary to achieve the desired compression. *Cohesiveness* (A_2/A_1 , where A_2 is the area under the force deformation curve for the compression cycle of the second bite and A_1 is the corresponding area for the first bite), *Gumminess*, product of hardness and cohesiveness, *Springiness* (DD_2 , the height in mm of the sample recovery during the time that elapsed between

the end of the first bite and the start of the second bite), *Chewiness* (the product of gumminess and springiness in N-mm) and *Degree of Elasticity* (DU_1/DD_1 , the ratio of the recovery time to the time for deformation during the first compression, and *Adhesiveness* the negative force (N) resulting from product adhesion to the instrument sample plates.

Other Measurements

Water-holding capacity was determined by subjecting a 5 mm slice of the gel to a 500g static force for approximately 30 min. Water holding capacity is presented as the ratio of the amount of water expressed to the total weight of the sample. The **fold test**, used in evaluating surimi products (Suzuki, 1981), evaluates the resilience of the gel by observing the tendency to crack by folding twice. Scoring is as follows: AA, double fold, no crack; A, single fold, no crack; B, single fold, partial crack; C, single fold, total crack; D, single fold, total break. **Solvation** was the g of the water retained per g of sample after centrifugation of the protein samples using:

$$S = (w_1 - w_2)/w_3 \quad (3)$$

w_1 = the wet solids plus the tube weight after centrifugation, w_2 = sample plus tube weight after lyophilization and w_3 = dry sample weight.

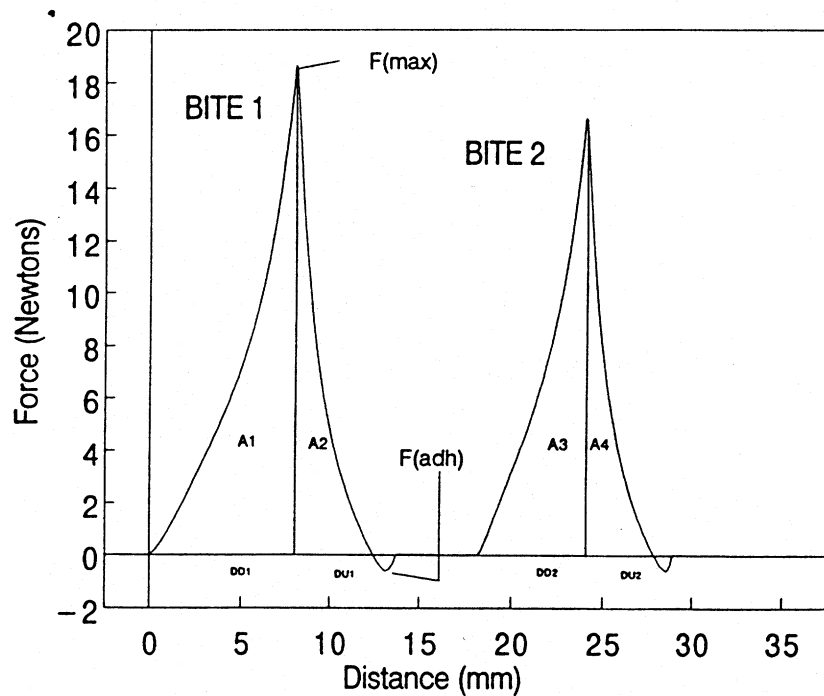


Figure 1—Typical texture profile analysis (TPA) curve.

RESULTS & DISCUSSION

Mixed Protein Gels

General Gel Characteristics. All gels created with the mixtures of casein and other proteins were opaque. The randomness of aggregation determines the relatively high concentration required for gelation. The casein/casein formulations produced very weak gels, primarily due to the heat stability of the casein, which is attributable to the lack of a secondary and tertiary structure. Due to the lack of a solid-like structure, texture profile analyses of these gels were not conducted. The casein/egg albumin and the casein/whey formulations did, however, result in firm gels.

Solvation. The water binding of protein gels is influenced by the factors that control texture. Data for the solvation characteristics of the protein gels are shown in Table 1.

Table 1—Solvation Characteristics of Protein Gels Containing 2.5% Casein or More

		Calcium Ion Concentration (mM)					
Protein Addition	Protein Concentration (%)	10	20 No Rennet	40	10	20 Rennet	40
g water/g protein							
Casein	5	3.48	3.17	2.18	3.30	2.91	1.51
EA	5	3.14	2.58	1.90	2.47	1.98	1.73
WPC	5	2.49	1.90	1.68	2.05	1.57	1.37
Casein	7.5	3.67	2.91	2.37	4.00	3.14	2.77
EA	7.5	2.88	2.47	1.93	2.78	2.10	1.78
WPC	7.5	2.56	1.87	1.71	2.18	1.47	1.38
Casein	10	3.27	2.90	2.33	3.85	3.49	3.02
EA	10	3.17	2.50	1.98	2.76	2.16	1.80
WPC	10	2.10	1.97	1.78	2.42	1.65	1.33
Kamaboko		2.07					

EA = Chicken Egg Albumin, WPC = Whey Protein Concentrate

At lower calcium concentration (CC), binding of calcium decreases, and the solvation increases. Behavior was similar for WPC and EA gels. Competitive binding generally occurs among water, salt, and amino acid side groups. At higher salt concentrations, interactions of water and salt may predominate, yielding a "dehydrated" protein. Interactions among proteins and ionic strength appear to affect the water binding of the protein samples more than protein concentration (PC) as evidenced by the relatively small changes in solvation resulting from the PC. Degree of solvation was greatest in the casein (CN) + CN samples (CN + CN > CN + EA > CN + WPC) for each CC, within a given total PC. Because WPC is compact, an improvement in water binding was expected; however, heating, the presence of fat in the WPC, and the interaction with the CN resulted in a reduction in solvation. Rennet treatment reduced in solvation for the mixtures of CN + EA and CN + WPC but increased solvation for the CN + CN samples at the higher PC.

Textural Parameters. The textural parameters for the protein gels without rennet are shown for CN and EA in Tables 2 and 3.

Table 2—Texture Profile Analysis of Kamaboko and Gels with 2.5% Casein and Chicken Egg Albumin (EA) - No Rennet

Calcium Conc. (mM)	Protein Conc. (g/100g)	Hardness (N)	Cohesiveness	Springiness (mm)	DE
10	5	13.3	0.602	3.93	0.533
20	5	29.6	0.598	3.97	0.598
40	5	43.0	0.638	4.00	0.590
10	7.5	15.6	0.615	3.65	0.575
20	7.5	24.1	0.609	3.93	0.609
40	7.5	44.4	0.605	4.10	0.594
10	10	14.2	0.511	3.88	0.584
20	10	32.4	0.601	3.87	0.631
40	10	41.8	0.595	4.03	0.621
Kamaboko		5.9	0.752	4.60	0.800

DE = Degree of Elasticity

Table 3—Texture Profile Analysis of Kamaboko and Gels with 2.5% Casein and Chicken Egg Albumin (EA) - Rennet

Calcium Conc. (mM)	Protein Conc. (g/100g)	Hardness (N)	Cohesiveness	Springiness (mm)	DE
10	5	14.0	0.422	3.87	0.554
20	5	41.0	0.578	3.88	0.583
40	5	54.6	0.585	3.85	0.602
10	7.5	15.6	0.368	3.95	0.575
20	7.5	40.8	0.584	4.03	0.602
40	7.5	51.5	0.586	4.05	0.615
10	10	25.7	0.445	3.95	0.602
20	10	39.3	0.566	3.93	0.612
40	10	57.7	0.607	4.25	0.630
Kamaboko		5.9	0.752	4.60	0.800

DE = Degree of Elasticity

The gels with added EA were compressed to 50% of their original height without any evidence of yield whereas the gels with added WPC yielded at 50 % compression and are therefore not presented. The differences in the TPA responses of the gels with the addition of rennet may be due to major structural differences in the microstructure. Differences in the textural parameters between gels with and without rennet were apparent throughout. The effects of centrifugation/solvation on the textural properties were analyzed using an analysis of covariance with solvation as the covariate. The result was a small increase in hardness only (correlation: $R = 0.577$). Little or no effect was seen in other textural parameters.

Hardness. Gel hardness (F_{max}), as a function of CC, is shown in Figure 2. For the CN and EA gels without rennet, hardness was greatest at the highest ionic strength and was dependent solely on CC.

Cohesiveness and Gumminess. Cohesiveness measures the ability of a material to stick to itself. The failure of the CN + WPC gels makes the cohesiveness values for these gels somewhat questionable. The average CV of the cohesiveness of the CN + WPC gels was 24 and 22% for gels with and without rennet respectively, compared with 6.2 and 8.9% for the CN + EA gels. Clearly, cohesiveness, especially at low CC, is reduced by the addition of rennet. Cohesiveness of all CN + EA gels without rennet was equal to or greater than that of gels with rennet.

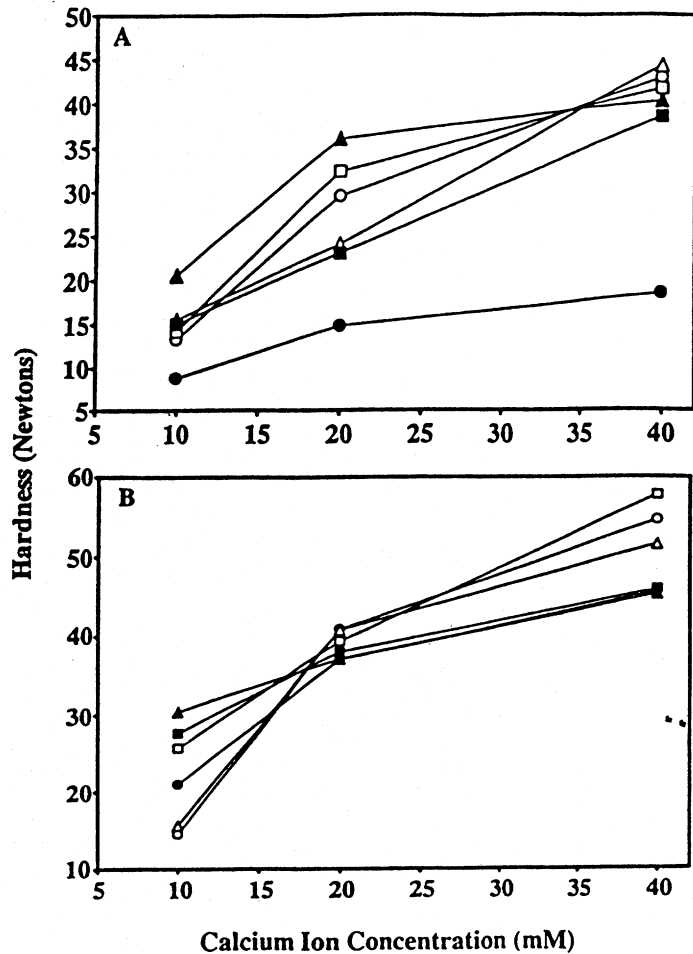


Fig. 2--Effect of Ca^{2+} concentration on hardness of casein gels with protein additives, without (A) and with (B) rennet. Chicken egg albumin \circ -5%; Δ -7.5%; \square -10%; whey protein concentrate, \bullet -5%; \blacktriangle -7.5%; \blacksquare -10%.

The cohesiveness was affected more by CC in the gels with added rennet. Increased hardness of the rennet gels was the predominant factor in their increased gumminess. Little differences resulted from added PC except at the highest concentration.

Springiness and Chewiness. Because the springiness of the CN + EA gels varied over a small range (from 3.65 to 4.25 mm), the resultant chewiness values were similar to those for gumminess; the gels containing unstable rennet showed the greatest chewiness.

Degree of Elasticity. The effect of rennet on the degree of elasticity of the protein gels, as a function of CC, is shown in Figure 3. Gel elasticity, with and without rennet, was dependent on protein type and PC. Without rennet, CN + EA gels showed maximal elasticity at higher CC and 10% added EA. The CN + WPC gels showed maximal elasticity at the higher PC, 10 mM Ca^{2+} . These gels were the most elastic of all those evaluated. When rennet was added, the result

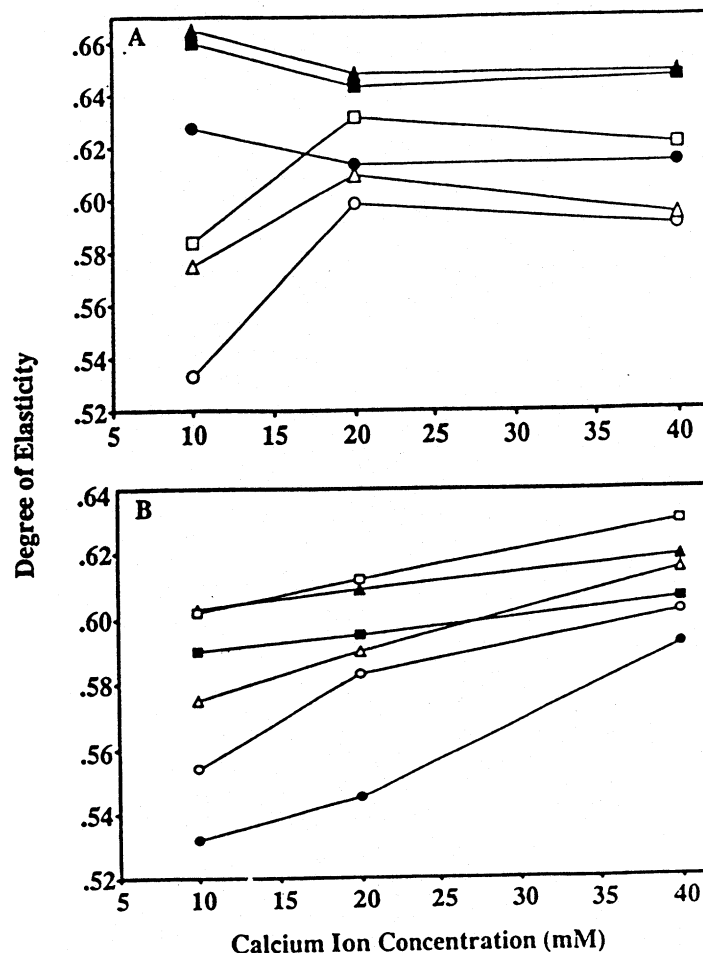


Fig. 3—Effect of Ca^{2+} concentration on degree of elasticity of casein (2.5%) gels with protein additives, without (A) and with (B) rennet. Chicken egg albumin ○ -5%; ▲ -7.5%; □ -10%; whey protein concentrate, ● -5%, ▲ -7.5%; ■ -10%.

The addition of EA to the CN yields a reasonably firm gel with good elastic and cohesive properties. However, additional formulation would be required to use these mixtures for surimi-like products.

Calcium Caseinate Gels

Phosphate Addition. A significant increase in the viscosity of calcium caseinate solutions was observed with the addition of phosphate (Konstance and Strange, 1991). Phosphate binds calcium and creates linkages that improve the gel matrix. The binding of Ca^{2+} to residues in a phosphate cluster could be strongly dependent on the extent of prior binding of Ca^{2+} in the region of that molecule. The use of the Glass H hexametaphosphate increases the available phosphate residues and improves binding. The calcium caseinate gels with 0.5% (wt/wt) and without added phosphate, were analyzed as controls at solids contents of 25, 35 and 45%. Table 4 shows the results of the rheological analysis and other parameters of these samples and those of the target material, kamaboko.

was an almost linear relationship with both the CN + EA and CN + WPC gels; elasticity was maximal at the highest PC and CC.

Comparison with Kamaboko. The solvation value of the Kamaboko was 2.07 grams of water per grams of dry solid. The CN + EA gels and the CN + WPC gels exhibit water binding characteristics that are as good as or better than those of the Kamaboko (Table 2). However, the CN + WPC gels have a texture that is too brittle for use in a surimi-like food without further formulation with hydrocolloids or polysaccharides. Despite the improved elasticity with 7.5 to 10% added WPC at 10 mM Ca^{2+} and without rennet, the tendency to failure and relatively poor cohesiveness appear to make them unsuitable for this use.

Table 4—Texture Parameters of Caseinate Gels with/without Phosphate

Solids (%)	P ^a	Hardness (N)	Cohesiveness	Elasticity	WHC ^b (g/g)
25	N	7.67	0.655	0.413	7.70
25	Y	13.24	0.680	0.456	6.80
35	N	10.18	0.661	0.420	1.01
35	Y	17.10	0.732	0.574	1.01
45	N	20.34	0.659	0.472	0.91
45	Y	29.13	0.699	0.576	1.19
Kamaboko	NA	18.53	0.752	0.795	1.99

^a Phosphate added as hexametaphosphate, Yes or No

^b WHC = Water Holding Capacity

Both the hardness and degree of elasticity of the calcium caseinate gels increased with solids content and the addition of phosphate, but cohesiveness was dependent only on the added phosphate. Water holding capacity improved at caseinate concentrations greater than 25%. When compared with kamaboko, the caseinate gels compared favorably in terms of hardness, cohesiveness and water holding capacity. The degree of elasticity in the gels with added phosphate increased but they were not as elastic as the kamaboko.

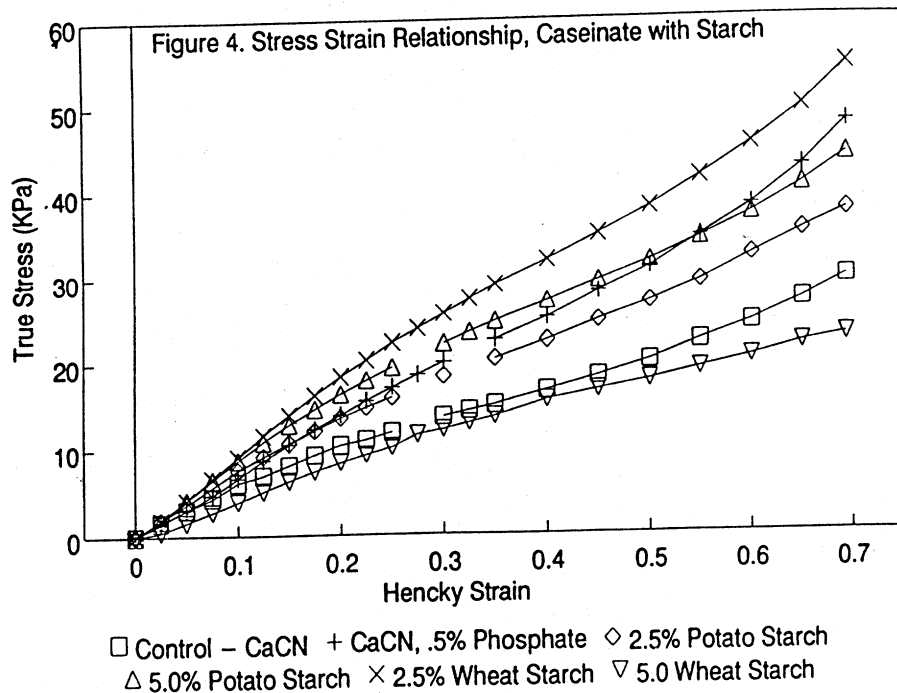
Table 5—Effect of Starches on Textural Properties (Calcium Caseinate with Phosphate)

Sample	Add. ^a	Hard. ^b	Cohes. ^c	Elast. ^d	Spring. ^e	Adh. ^f
Control	NA	17.10	0.73	0.57	5.97	0.18
w/PS	2.5	12.49	0.68	0.52	5.51	0.25
w/PS	5.0	15.91	0.65	0.50	4.82	0.34
w/WS	2.5	17.70	0.72	0.59	5.62	0.29
wWS	5.0	15.59	0.70	0.54	5.55	0.39
Kamaboko		18.53	0.75	0.80	6.77	0.10

^aPercent addition; ^bHardness (N); ^cCohesiveness; ^dDegree of Elasticity; ^eSpringiness (mm); ^fAdhesiveness (N); PS- -Potato Starch; WS- -Wheat Starch

Starch Addition. The gelatinization properties of starches are well known. Starches should increase gel strength and elasticity through a composite reinforcing effect and water binding. The evaluation of the textural parameters with added food grade starch (unmodified potato and wheat) is shown in Table 5. Sample hardness, springiness and degree of elasticity were effectively unaltered by the addition of starch; however, the cohesiveness, when compared to the control, was reduced.

The stress-strain curves for the calcium caseinate gels with and without added starch are shown in Fig. 4. The sigmoid shape of the curves is indicative of a gel that is predominantly yielding (concave downward portion) in the Hencky strain range of 0.1 to 0.4 and a predominantly compressible material (concave upward portion) with Hencky strain range of 0.4 to 0.7. The addition of 2.5% potato and wheat starch, 5% potato starch and phosphate resulted in an increase in the Apparent Young's Modulus (the slope of the initial linear portion of the stress-strain relationship) compared to that of the control sample. The gels made with 5% wheat starch were less firm and less elastic, possibly indicating an extensive swelling of the starch



granules (Whistler and Daniel, 1985) and a disruptive effect on the gel matrix. Although the stress-strain curves showed firmer gels with the addition of starch, the textural modifications that are considered to be advantageous in creating a surimi-like product were not evident.

Carrageenan Addition. Carrageenans, especially κ -carrageenan, are frequently added to dairy products. Carrageenans behave like casein in that they require heat to bring them into solution or dispersion, and they gel upon cooling. The commercially important interaction of casein with milk proteins is an example of the electrostatic interaction that can occur between the negatively charged carrageenans and the positively charged sites on the proteins. Binding occurs at the point at which they interact to form a complex. Binding with other caseins, is however weaker. Texture parameters of the calcium caseinate gels are in Table 6.

Table 6—Effect of κ -Carrageenan on Textural Properties

Sample	Hard. ^b	Cohes. ^c	Elast. ^d	Spring. ^e	WHC ^f	Fold ^g	Adhes. ^h
Control ⁱ	17.10	0.73	0.57	5.97	1.01	A	0.181%
κ -carr	20.27	0.70	0.53	4.96	0.89	AA	0.442%
κ -carr	23.45	0.76	0.62	6.24	0.91	AA	0.27
3% κ -carr	25.59	0.72	0.62	6.38	1.93	A	0.15
4% κ -carr	28.56	0.76	0.64	6.42	1.48	A	0.21
1% κ -carr	16.69	0.70	0.75	7.41	0.80	AA	0.20
Kamaboko	18.53	0.75	0.80	6.77	1.99	AA	0.10

^apH 6.4, 18 hr cook; ^bHardness in Newtons; ^cCohesiveness; ^dDegree of Elasticity;

^eSpringiness in mm; ^fWater Holding Capacity; ^gFoldability; ^hAdhesiveness; ⁱControl sample is gel from calcium caseinate with phosphate added.

The gels made with added carrageenan were all harder than the control sample. Cohesiveness, springiness and elasticity were not affected with the addition of 1% carrageenan, but all increased at carrageenan concentration equal to or greater than 2%.

All samples studied showed better water-binding characteristics when compared to kamaboko, and all but the samples with the higher concentrations of κ -carrageenan exhibited foldability equivalent to the target material. All samples were, however, slightly more adhesive than the kamaboko.

CONCLUSIONS

The addition of proteins, such as egg albumin and whey protein concentrate to casein along with calcium and rennet, significantly alters the functional properties. The ion concentration and the protein type are the major contributors to these changes in functionality. The protein concentration at the amounts studied, although it shows some impact on gel elasticity, plays a much smaller role. The addition of rennet to these protein systems reduces water-binding characteristics, as measured by solvation, while significantly increasing the hardness, cohesiveness and gumminess of the casein/egg albumin gels. The addition of whey generally weakens gels that yield when subjected to a compressive strain of 50%. As with the mixed protein gels, the addition of carrageenan to calcium caseinate offers a variety of textural properties. Phosphate addition is critical to the creation of firm gels while adding >2% κ -carrageenan results in significant improvement in gel cohesiveness, springiness, water holding capacity and degree of elasticity.

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